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Trichromacy in Australian Marsupials

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Summary

Vertebrate color vision is best developed in fish, reptiles, and birds with four distinct cone receptor visual pigments [1, 2]. These pigments, providing sensitivity from ultraviolet to infrared light, are thought to have been present in ancestral vertebrates [3]. When placental mammals adopted nocturnality, they lost two visual pigments, reducing them to dichromacy; primates subsequently reevolved trichromacy [4]. Studies of mammalian color vision have largely overlooked marsupials despite the wide variety of species and ecological niches and, most importantly, their retention of reptilian retinal features such as oil droplets and double cones [5]. Using microspectrophotometry (MSP), we have investigated the spectral sensitivity of the photoreceptors of two Australian marsupials, the crepuscular, nectivorous honey possum (*Tarsipes rostratus*) and the arhythmic, insectivorous fat-tailed dunnart (*Sminthopsis crassicaudata*); these species are representatives of the two major taxonomic divisions of marsupials, the diprotodonts and polyprotodonts, respectively. Here, we report the presence of three spectrally distinct cone photoreceptor types in both species. It is the first evidence for the basis of trichromatic color vision in mammals other than primates. We suggest that Australian marsupials have retained an ancestral visual pigment that has been lost from placental mammals.

Results and Discussion

The spectral sensitivity of both single and double cones, and their associated oil droplets, was sampled in the retinae of three honey possums and three fat-tailed dunnarts. The mean wavelengths of maximum absorbance (λ_{max}) of the cone visual pigments were found at 557, 505, and approximately 350 nm in the honey possum and at 535, 509, and approximately 350 nm in the fat-tailed dunnart, representing sensitivity to long (LWS), medium (MWS), and ultraviolet (UVS) wavelengths, respectively (Figure 1). The LWS and MWS visual pigments were found in either single or double cones, but not as LWS/MWS pairings. UVS records were only from single cones. Oil droplets were found to be transparent in both species, as indicated by their negligible absorbance ($>$0.002) between 350 nm and 750 nm. Therefore, it is likely that oil droplets gather and focus light onto the cone outer segments rather than act as spectral filters. The spectral absorbance of rods, photoreceptors subserving primarily night vision, was also determined. The λ_{max} of the rod visual pigment in the honey possum was located at 502 nm (Figure 1E), similar to 501 nm in another diprotodont marsupial, the tammar wallaby (*Macropus eugenii*) [6]. The λ_{max} of the rod was at 512 nm in the fat-tailed dunnart (Figure 1E′).

We report that, in both honey possum and fat-tailed dunnart, MWS cones and rods possess similar spectral sensitivity and photochemical properties, with a post-bleach build-up of photoproduct that absorbed below 430 nm (Figures 1C and C′). A similar λ_{max} between MWS cones and rods, also reported in birds [7–9], would allow for optimal function in the midspectral range at different levels of illumination. Both species of marsupial are active during the day and after dark [10, 11].

The spectral sensitivity of the cone visual pigments in both the honey possum and fat-tailed dunnart can also be correlated with the specific requirements of their visual ecology. In the honey possum, LWS cones confer sensitivity in the yellow-red region of the spectrum, possibly subserving the detection of yellow and red nectar-producing flowers, particularly in a crepuscular light environment, where maximum contrast is generated by yellow and maximum brightness is generated by red [12]. Combined with other cone types, LWS cones may allow animals to assess the maturity of flowers, which are yellow or red when ripe but green when unripe. By contrast, in the fat-tailed dunnart, LWS cones are maximally sensitive in the green-yellow region of the spectrum (Figure 2). Combined with MWS cones, LWS cones could form the basis for discrimination of cryptically colored green and brown prey, such as insects or small reptiles.

Our study, the first to examine marsupial cones using MSP, will need to be extended to determine whether three visual pigments are present in other Australian marsupials; immunohistochemical studies, such as those showing two visual pigments in tammar wallaby, may leave pigment classes undetected [13]. However, the similarity of our MSP data for a diprotodont, the honey possum, and a polyprotodont, the fat-tailed dunnart, indicates that the presence of three visual pigments is a feature spanning the marsupial subclasses.

Five classes of vertebrate visual pigments, classified according to the molecular structure of opsins, are found among fish, birds, and reptiles. There are four cone classes, M/LWS, SWS1, SWS2, and RH2, and a rod class, RH1 [3, 14], all thought to be present in ancestral vertebrates [2]. SWS2 and RH2 have been lost from placental mammals, while primates reevolved trichromacy from duplication of the M/LWS gene [4]. Given the close molecular homology between RH1 and RH2 opsins [3, 7, 14], and the similarity of spectral absorbance and photochemical properties of MWS cone and rod visual pigments, it seems possible that, as in birds [1, 7, 15], the marsupial MWS cone visual pigment belongs to the RH2 class. If the presence of the RH2

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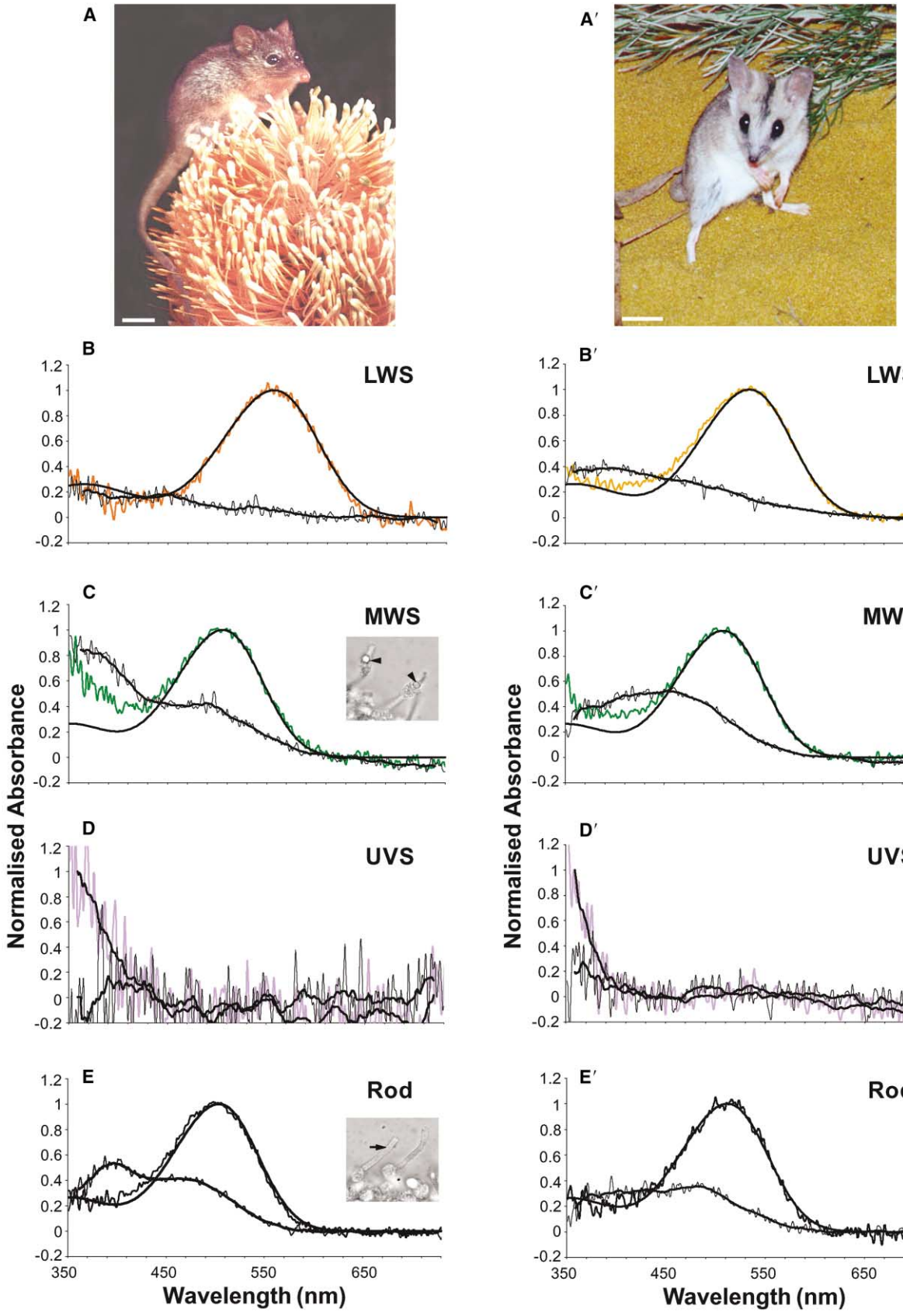
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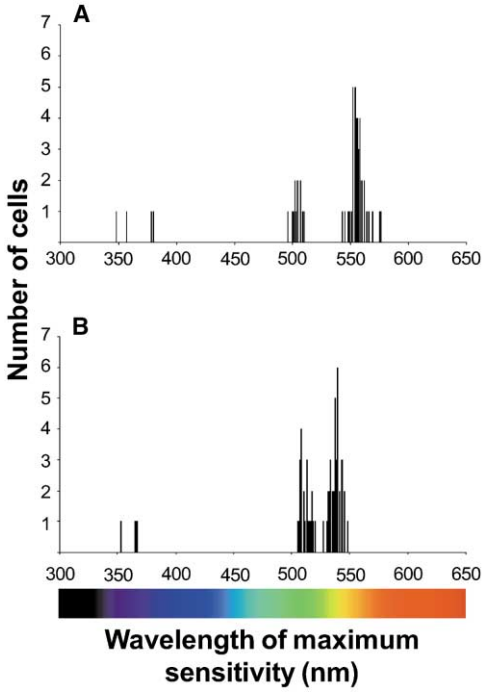

Figure 2. Distribution of Cone Visual Pigments in the Two Marsupials

(A and B) Frequency histograms showing the distribution of the λ_{max} from individual records from cones in the (A) honey possum and the (B) fat-tailed dunnart.

gene in marsupials is confirmed by sequence analysis, the implication is that the gene has been retained from the ancestral reptilian retinal design. Alternatively, a duplication of MWS/LWS may have taken place, as was the case in primates.

Disparate selective pressures for placentals and Australian marsupials, early in mammalian evolution, may underlie the differing outcomes in terms of the number of cone classes in their retina. Placental mammals avoided predation and competition by adopting nocturnality, reducing the requirement for discrimination across the visual spectrum. By contrast, Australian marsupials, isolated from predation by large diurnal species [16], may have retained activity patterns encompassing diurnality. If so, color discrimination beyond dichromacy was presumably advantageous. However, American marsupials would have experienced similar evolutionary pressures to their placental counterparts, since the two groups were sympatric. Although an immunohistochemical study of the South American opossum (*Didelphis marsupialis aurita*) revealed the presence of only two cone classes [17], MSP analysis is essential to determine the presence of two or three cone visual pigments in their retina.

In conclusion, we suggest that the presence of MWS cones in the retina of the honey possum and fat-tailed dunnart is a feature retained from the ancestral reptilian retinal design. Consequently, the potential for trichromacy in marsupials may have a different evolutionary origin from that in primates. Subsequent behavioral and molecular biological studies will determine whether trichromacy and its use are general features of the marsupial retina and may throw light on the evolutionary origins.

Experimental Procedures

Spectral sensitivity was determined by scanning the outer segments of individual photoreceptors, and the associated oil droplets of cones, using a microspectrophotometer [18]. Cones were readily distinguished from rods by their wide inner segments containing a highly refractile oil droplet (see photomicrograph insets of MSP preparations, Figures 1C and 1E). The rod outer segments were cylindrical and substantially longer than those of the cones. Microscope optics pass a beam (2×2 μm) of monochromatic light through a cell and measure the amount of light absorbed at each wavelength between 350 nm (ultraviolet) and 750 nm (far-red). Cells were sampled from across the entire retina to minimize the possibility of omitting a type of photoreceptor or oil droplet. The average scans for each spectral cell type are presented as normalized curves with best-fitted retinal (A_1) visual pigment templates calculated from the equations of Govardovskii et al. [19]. Following bleaching of the visual pigment, the cells were rescanned, and the average post-bleach spectra, with their running averages, were used for presentation (see Figure 1). For the UVS pigments, values of λ_{max} were approximated. The study was approved by the Animal Ethics and Experimentation Committee, University of Western Australia. Honey possum were collected under license from the Department of Conservation and Land Management, Western Australia. The fat-tailed dunnarts were obtained from an established breeding colony at the University of Adelaide, South Australia.

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Figure 1. Spectral Absorbance of Visual Pigments in the Honey Possum and Fat-Tailed Dunnart

(A) The nectivorous diprotodont honey possum.

(A′) The insectivorous/carnivorous polyprotodont fat-tailed dunnart.

(B–E) Normalized averaged absorbance spectra of visual pigments in the retina of the honey possum.

(B′–E′) Normalized averaged absorbance spectra of visual pigments in the retina of the fat-tailed dunnart.

Honey possum: (B) LWS cones (n = 39, average maximum optical density (OD) = 0.009); (C) MWS cones (n =16, OD = 0.016); (D) UVS cones (n = 4, OD = 0.005); and (E) rods (n = 26, OD = 0.021), with λ_{max} at 557 nm, 505 nm, approximately 350 nm, and 502 nm, respectively.

Fat-tailed dunnart: (B′) LWS cones (n = 38, OD = 0.016); (C′) MWS cones (n = 20, OD = 0.019); (D′) UVS cones (n = 3, OD = 0.02); and (E′) rods (n = 13, OD = 0.012), with λ_{max} at 535 nm, 509 nm, approximately 350 nm, and 512 nm, respectively.

Each graph shows the average pre-bleach spectra (upper traces) with best-fitted visual pigment templates (solid lines) and average post-bleach spectra (lower traces) with their running averages (solid lines). Because the average pre-bleach spectrum of the UVS cones was not fitted well by the ultraviolet visual pigment template, its running average absorbance is displayed. All cone inner segments possessed an oil droplet (arrowheads, photomicrograph inset in [C]) with a diameter of approximately 2 μm. Rods lacked an oil droplet and had substantially longer outer segments (arrow, photomicrograph inset in [E]). The scale bars in (A) and (A′) represent 1 cm.

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References

1. Okano, T., Kojima, D., Fukada, Y., Shichida, Y., and Yoshizawa, T. (1992). Primary structures of chicken cone visual pigments: vertebrate rhodopsins have evolved out of cone visual pigments. Proc. Natl. Acad. Sci. USA *89*, 5932–5936.
2. Bowmaker, J.K. (1998). Evolution of colour vision in vertebrates. Eye *12*, 541–547.
3. Yokoyama, S. (2000). Molecular evolution of vertebrate visual pigments. Prog. Retin. Eye Res. *19*, 385–419.
4. Jacobs, G.H. (1993). The distribution and nature of colour vision among mammals. Biol. Rev. *68*, 413–471.
5. Ahnelt, P.K., and Kolb, H. (2000). The mammalian photoreceptor mosaic-adaptive design. Prog. Retin. Eye Res. *19*, 711–777.
6. Hemmi, J.M., Maddess, T., and Mark, R.F. (2000). Spectral sensitivity of photoreceptors in an Australian marsupial, the tammar wallaby (*Macropus eugenii*). Vision Res. *40*, 591–599.
7. Bowmaker, J.K., Heath, L.A., Wilkie, S.E., and Hunt, D.M. (1997). Visual pigments and oil droplets from six classes of photoreceptor in the retina of birds. Vision Res. *37*, 2183–2194.
8. Das, D., Wilkie, S.E., Hunt, D.M., and Bowmaker, J.K. (1999). Visual pigments and oil droplets in the retina of a passerine bird, the canary *Serinus canaria*: microspectrophotometry and opsin sequences. Vision Res. *39*, 2801–2815.
9. Hart, N.S. (2000). The visual ecology of avian photoreceptors. Prog. Retin. Eye Res. *20*, 675–703.
10. Arrese, C., Dunlop, S.A., Harman, A.M., Braekevelt, C.R., Ross, W.M., Shand, J., and Beazley, L.D. (1999). Retinal structure and visual acuity in a polyprotodont marsupial, the fat-tailed dunnart (*Sminthopsis crassicaudata*). Brain Behav. Evol. *53*, 111–126.
11. Arrese, C., Archer, M., and Beazley, L.D. (2002). Visual capabilities in a crepuscular marsupial, the honey possum (*Tarsipes rostratus*): a visual approach to ecology. J. Zool. (Lond.) *256*, 151–158.
12. Endler, J.A. (1993). The color of light in forests and its implications. Ecol. Monograph. *63*, 1–27.
13. Hemmi, J.M., and Grunert, U. (1999). Distribution of photoreceptor types in the retina of a marsupial, the tammar wallaby (*Macropus eugenii*). Vis. Neurosci. *16*, 291–302.
14. Ebrey, T., and Koutalos, Y. (2000). Vertebrate photoreceptors. Prog. Retin. Eye Res. *20*, 49–94.
15. Heath, L.A., Wilkie, S.E., Bowmaker, J.K., and Hunt, D.M. (1997). The rod and green cone opsins of two avian species, the budgerigar, *Melopsittacus undulatus*, and the mallard duck, *Anas platyrhynchus*. Gene *204*, 121–126.
16. Archer, M. (1984). Vertebrate Zoogeography and Evolution in Australasia (Carlisle, NSW, Australia: Hesperian Press).
17. Ahnelt, P.K., Hokoç, J.N., and Röhlich, P. (1995). Photoreceptors in a primitive mammal, the South American opossum, *Didelphis marsupialis aurita*: characterisation with anti-opsin immunolabelling. Vis. Neurosci. *12*, 793–804.
18. Shand, J., Archer, M.A., Thomas, N., and Cleary, J. (2001). Retinal development of West Australian dhufish, *Glaucosoma hebraicum*. Vis. Neurosci. *18*, 711–724.
19. Govardovskii, V.I., Fyhrquist, N., Reuter, T., Kuzmin, D.G., and Donner, K. (2000). In search of the visual pigment template. Vis. Neurosci. *17*, 509–528.